Euphytica (2006) 149: 145–159
DOI: 10.1007/s10681-005-9062-7

Quantitative trait locus analysis of wheat quality traits*

James C. Nelson[1,], Cristina Andreescu[1], Flavio Breseghello[3], Patrick L. Finney[2], Daisy G. Gualberto[2], Christine J. Bergman[2], Roberto J. Peña[4], Marie Reine Perretant[5], Philippe Leroy[5], Calvin O. Qualset[6] & Mark E. Sorrells[3]

[1]*Department of Plant Pathology, 4024 Throckmorton Hall, Kansas State University, Manhattan, KS 66506, USA;* [2]*US Department of Agriculture, Agricultural Research Service, Soft Wheat Regional Quality Laboratory, Ohio Agricultural Research and Development Center, Ohio State University, 1680 Madison Ave., Wooster, OH, 44691, USA;* [3]*Department of Plant Breeding and Genetics, 240 Emerson Hall, Cornell University, Ithaca, NY, 14853, USA;* [4]*International Maize and Wheat Improvement Center (CIMMYT, Int.), Apartado Postal 6-641, Delegación Cuauhtémoc, 06600, Mexico, D.F. Mexico;* [5]*UMR 1095 INRA-UBP Amélioration & Santé des Plantes, 234 Avenue du Brézet, F-63100 Clermont-Ferrand, France;* [6]*Genetic Resources Conservation Program, University of California, One Shields Avenue, Davis, California 95616-8602, USA*

Received 19 May 2005; accepted 22 November 2005

Key words: ITMI, quality, QTL, recombinant inbred, wheat

Summary

Milling and baking quality traits in wheat (*Triticum aestivum* L.) were studied by QTL analysis in the ITMI population, a set of 114 recombinant inbred lines (RILs) generated from a synthetic-hexaploid (W7985) × bread-wheat (Opata 85) cross. Grain from RILs grown in U.S., French, and Mexican wheat-growing regions was assayed for kernel-texture traits, protein concentration and quality, and dough strength and mixing traits. Only kernel-texture traits showed similar genetic control in all environments, with Opata *ha* alleles at the hardness locus *Ha* on chromosome arm 5DS increasing grain hardness, alkaline water retention capacity, and flour yield. Dough strength was most strongly influenced by Opata alleles at 5DS loci near or identical to *Ha*. Grain protein concentration was associated not with high-molecular-weight glutenin loci but most consistently with the *Gli-D2* gliadin locus on chromosome arm 6DS. In Mexican-grown material, a 2DS locus near photoperiod-sensitivity gene *Ppd1* accounted for 25% of variation in protein, with the *ppd1*-coupled allele associated with higher (1.1%) protein concentration. Mixogram traits showed most influence from chromosomal regions containing gliadin or low-molecular-weight glutenin loci on chromosome arms 1AS, 1BS, and 6DS, with the synthetic hexaploid contributing favorable alleles.

Some RI lines showed quality values consistently superior to those of the parental material, suggesting the potential of further evaluating new combinations of alleles from diploid and tetraploid relatives, especially alleles of known storage proteins, for improvement of quality traits in wheat cultivars.

Abbreviations: AWRC, alkaline water retention capacity; H(L)MW, high(low)-molecular-weight; ITMI, International Triticeae Mapping Initiative; PAGE, polyacrylamide gel electrophoresis; RIL, recombinant inbred line; RFLP, restriction fragment length polymorphism; RI, recombinant inbred; SDS, sodium dodecyl sulfate; NIR, near-infrared reflectance; SE, softness equivalent

Introduction

Bread wheat (*Triticum aestivum* L.), one of the staple cereals of humankind, is usually eaten in the form of

*Contribution number 06-77J from the Kansas Agricultural Experimental Station.

baked products. Flour milled from the wheat grain is mixed with water, salt, and often leavening and other ingredients and then baked or otherwise cooked. The processing and end-use characteristics of the grain, collectively known as quality traits, are under genetic and environmental control. Molecular-genetic studies to elucidate this control may increase the efficiency of breeding wheat for improved quality.

Prospective cultivars in wheat breeding programs are subjected to an array of quality tests. For bread wheat, these tests typically measure flour yield, protein concentration and composition, kernel texture, and dough mixing properties, and may also measure properties of baked goods such as loaf volume and crumb structure. However, what constitutes desirable quality depends on the intended use of the grain. For example, higher protein concentration, alkaline water retention capacity (AWRC), and dough strength are desired for breads but not for cakes and cookies (biscuits).

The measurable physicochemical properties underlying quality may be grouped into kernel hardness and dough stiffness, strength, extensibility, and stability (Konzak, 1977). Stiffness or viscosity is resistance to deformation and may be expressed in mechanical terms as work or energy. Tenacity, a property referred to below, is expressed as the maximum force required for deformation of a dough. Strength is the persistence of viscosity upon stretching, expressed mechanically as the (inverse) rate of decline of deformation force with extension. Extensibility is expressed as the distance to which dough extends before breaking. Stability or mixing tolerance is the persistence of viscosity with continued mixing of the dough.

Physical and handling properties of dough are evaluated by various means. Mixograph tests, recording a time curve of dough resistance to mixing, are used by breeders for selection as well as by bakers for predicting dough-handling requirements. In the Pelshenke test, a ball of leavened whole-meal dough is placed in 30°C water and strength is assessed as time to disintegration. The Zeleny and sodium dodecyl sulfate (SDS)-sedimentation tests exploit the positive correlation between protein quality (expressed as quantity and composition of water-insoluble glutenins) and the settling volume of a shaken whole-meal suspension in a solution of lactic acid and SDS, respectively. Dough viscoelastic properties can also be measured with an alveograph, a machine that records the pressure during expansion of a bubble blown from a disc of dough. These tests provide small-sample predictors of loaf volume, which may be measured directly along with other parameters in later breeding generations when larger grain samples are available for a baking test.

The protein composition of the wheat grain is central to the assessment of quality. Wheat is unique among cereals in containing large quantities of gluten, a complex of proteins giving dough the capacity of retaining the CO_2 bubbles that allow leavened bread to rise. Gluten may be chemically partitioned into gliadins, important in dough viscosity and extensibility, and glutenins, considered to affect dough strength and elasticity and thus loaf volume. While studies have suggested the involvement of most wheat chromosomes in control of protein or processing quality (Konzak, 1977; Mansur et al., 1990), the HMW glutenin and gliadin seed storage proteins have provided the genetic markers easiest to assay, most predictive of breadmaking quality, and consequently the subjects of most genetic studies of quality. Fractionation and electrophoresis of protein extracts from aneuploid stocks and substitution lines (Wrigley & Shepherd, 1973) showed the gliadins to be products of genes lying on the short arms of chromosomes in wheat homoeologous groups 1 and 6. Individual electrophoretic gliadin bands have been associated with dough strength (du Cros et al., 1983; Branlard and Dardevet, 1985a). Association of breadmaking quality with HMW glutenin subunits encoded by genes on the long arms of chromosomes 1D and 1B has been well established (Payne et al., 1981), and individual bands detected with SDS polyacrylamide gel electrophoresis (PAGE) are used as selection criteria in conventional breeding programs.

Studies in panels of unrelated wheat lines (Payne et al., 1981; Branlard & Dardevet, 1985ab; Campbell et al., 1987) and in progeny of single crosses (Nieto-Taladriz et al., 1994; Payne et al., 1987; Rousset et al., 1992) show associations of individual electrophoretic bands (notably the *Glu-B1* 5 + 10 combination) with quality traits such as loaf volume and dough stiffness. Still, these associations predict only a limited range of quality traits (Cressey et al., 1987), and with unsatisfactory consistency owing to epistatic interactions among the many gluten protein loci (Rousset et al., 1992; Nieto-Taladriz et al., 1994). Weegels et al. (1996) proposed that glutenin macropolymer quantity influences quality more than does HMW subunit composition. However important to breadmaking quality may be the composition of endosperm proteins, it is likely that other genes, some highly influenced by environment, regulate the synthesis and storage of these proteins.

Increased kernel hardness requires higher milling energy, but results in higher flour yield (Symes, 1965,

Bassett et al., 1989) and better flowing and sifting properties during milling (Pomeranz & Williams, 1990). The major determinant of hardness is the *Ha* locus on chromosome arm 5DS, carrying two tightly linked puroindoline genes, *PinA* and *PinB* (reviewed in Martin et al., 2001). The hardness allele of *PinB*, though leading to a softer kernel than the hardness (null) allele of *PinA*, nonetheless gave higher flour yield in inbred progeny of a cross between the two (Martin et al., 2001). The main effect of hardness on breadmaking qualities is attributed to higher starch damage during milling. This damage increases both water absorption and hydrolysis of starch into fermentable sugars that contribute to loaf volume (Pomeranz & Williams, 1990). Soft wheats tend to have lower protein and gliadin and glutenin alleles conferring reduced dough strength, which together with the reduced starch damage is thought to favor pastry products with desirable tenderness, dough spread, and a reduced affinity for water that minimizes baking time (Gaines et al., 1996). Measurements of hardness indicative of end-use properties are generally made by near-infrared reflectance (NIR) spectrometry, particle size in milling fractions (Finney & Andrews, 1986), or pearling index (Carrillo et al., 1990; & earlier literature).

While early-generation quality assays can indicate the potential quality of a wheat line in the test environment, quality traits are under strong environmental influence (McGuire & McNeal, 1974; Bassett et al., 1989). The use of DNA markers to refine understanding of environmental interactions with genes affecting quality should increase breeders' ability to design cultivars for target environments. There is also increasing interest in *Aegilops tauschii*, the diploid D-genome donor to hexaploid bread wheat, as a source of quality traits. The importance of the D genome to bread quality has long been known (Kerber & Tipples, 1969), and marker analysis offers the prospect of characterizing and transferring influential gene complexes more precisely from this source than is possible by conventional means. Some of this increased precision, and consequent economy, will come from the identification of the key genes/QTLs whose expression is being measured, perhaps indirectly, by different tests.

A few QTL analyses of wheat quality traits have been reported. Kernel hardness is invariably found to be strongly associated with the *Ha* gene (Sourdille et al., 1996; Igrejas et al., 2002), with other genomic regions contributing lesser effects. Markers for this locus also strongly influenced the closely related parameters flour yield, starch damage, AWRC, dough water absorption, and cookie diameter (Campbell et al., 1999) and, in a study by Perretant et al. (2000), governed dough strength as alveogram *W*. Mixing characters (peak height, tolerance, and mixing time) were dominated by HMW glutenin alleles on group-1 chromosomes (Campbell et al. 2001). However, dough strength in the cv. Courtot × Chinese Spring doubled-haploid progeny studied by Perretant et al. (2000) was not as strongly associated with HMW glutenin loci, perhaps because only those on chromosome 1A carried different alleles in the parents. Grain protein concentration has been associated with gliadin-linked loci on 1A (Campbell et al., 2001) and 6A (Perretant et al., 2000) and other loci not associated with known storage proteins in hexaploid and tetraploid wheats (Campbell et al., 2001; Perretant et al., 2000; Groos et al., 2003; Prasad et al., 2003; Blanco et al., 2002; Olmos et al., 2003).

The ITMI recombinant inbred lines (RILs) were developed and densely genotyped (Nelson 1995ab, Röder et al., 1998; Mingeot & Jacquemin, 1999; and others) and their seeds made available to researchers for QTL mapping. More than a hundred quality evaluations of subsets of the lines were contributed by several ITMI collaborators in this study over several years. The purpose was to use these data to identify regions of the wheat genome affecting milling and baking quality.

Materials and methods

Germplasm and growing conditions

Analyses were performed on 114 F_8-derived RILs developed from a cross between the synthetic hexaploid wheat line WPI 219 (also known as W7985 and as M6) and the spring wheat cultivar Opata 85; these parental lines are abbreviated below as M6 and Opata. The A and B genomes of the synthetic M6 were derived from a *T. turgidum* cultivar originally given as Altar 84 and its D genome was derived from an accession of *Aegilops tauschii* Coss.; these lines will be abbreviated here as TT and AeT. The grain samples tested were from 56 lines grown at Ithaca, New York (42.5° N, 76.5° W, 335 m above sea level, average annual wheat yield ~3.6 Mg ha^{-1}), in 1994; 89 lines grown at Tulelake, California (42 N, 121.5° W, 1230 m, ~5.7 Mg ha^{-1}) in 1995, 1996, and 1999; 86 lines grown at Clermont-Ferrand, France (46° N, 3° E, 330 m, ~7.4 Mg ha^{-1}) in 1994 and 1997; and 114 lines grown at Ciudad Obregón,

Sonora, Mexico (27° N, 110° W, 39 m, ~5.5 Mg ha^{-1}) in 1993–1994. Cropping conditions in all locations were suitable for full plant development and grain filling. Mexico and California samples were produced in a desert environment under optimal irrigation and fertilization.

Quality analyses

Analyses of U.S. grown grain samples were carried out at the United States Department of Agriculture (USDA) Soft Wheat Regional Quality Laboratory, in Wooster, Ohio, and those grown in Mexico and France at the respective wheat quality laboratories of the International Maize and Wheat Improvement Center (CIMMYT) in El Batán, Mexico and the National Institute for Agricultural Research (INRA) in Clermont-Ferrand, France. Depending on laboratory, tests were based on 25 to 50 g of grain. Both parents were also tested except in a few cases where seed of the synthetic parent was lacking. Samples were air-aspirated and, when necessary, hand-cleaned of foreign material and shriveled kernels. Analytical procedures for CIMMYT are detailed in Peña et al. (1990). For most tests the data used for QTL analysis were the means of two or more determinations within a site.

Flour protein concentration was determined by Kjeldahl procedure [AACC method 46-12, using boric acid modification (AACC, 1995)] at USDA, NIR (Technicon InfraAlyzer 350) calibrated against Kjeldahl at CIMMYT, and NIR (Percon Inframatic 8620) from 10g of wholemeal milled in a Cyclotec mill at INRA.

Grain hardness was measured by NIR at INRA, calibrated against hard and soft wheat standards, and as softness equivalent (SE) at USDA and CIMMYT. In the micromilling procedure for determination of SE, 25 g of grain ground in a Quadrumat Jr. mill were passed through three sets of breaking rolls and then dumped onto a 15.75-wire cm^{-1} (40 mesh) sieve above a 37-wire cm^{-1} (94 mesh) sieve. Because soft grain produces more fine material at the break, the overs of (weight of meal not passing through) the 94 mesh sieve are positively correlated with grain hardness. Softness equivalent (in percentage) is obtained by the standard formula

$$\mathrm{SE} = \frac{[(25 - \text{overs of } 40) - (\text{overs of } 94)] \times 100}{25 - \text{overs of } 40}$$

Flour yield was determined at USDA and INRA as the proportion by weight of straight-grade flour (the milled product after break and reduction steps) recovered from a milled grain sample, adjusted to correspond to a sample ground at 14% moisture. A further standard adjustment was applied at USDA to generate SE-adjusted yield, a convention based on the inverse relationship between softness equivalent and yield and their commonly observed variation in the same material grown in different environments.

Mixograms were obtained at USDA and INRA with the Swanson and Working Mixograph (National Mfg. Co., Lincoln, Nebraska, U.S.A.) following respectively Walker and Walker (1992) and AACC method 54-40 (AACC, 1995). The peak represents the point of maximum resistance to mixing, and the time to its occurrence (peak time) should be neither too long nor too short for good dough handling properties for pastry or bread end uses. The vertical width of the trace produced by the oscillating pen at any point indicates dough viscosity. This measurement was recorded as peak width, LOP width, and ROP width, where LOP and ROP refer to measurements taken at a set distance to left and right of peak, respectively; and tail width, measured also at a set distance on the right tail of the curve. Peak value, LOP value, and ROP value are the heights of the trace's midline at these points. These heights are also indicators of the viscosity of the dough and are, in practice, positively correlated with protein concentration. Measurements are also recorded in the ENV (envelope), a time period between ROP and tail regions. TIMEX represents the end of the indicated time period. Tail value indicates the total dough breakdown or loss of strength during the mixing cycle and tail slope indicates the rate of breakdown. Area scores for each stage of the mixing operation indicate the energy (in % torque min) expended during that stage.

In France the Pelshenke test and Zeleny sedimentation test and in Mexico a modified SDS-sedimentation test (Peña et al., 1990) were performed. At INRA and CIMMYT alveograms were recorded with the Chopin micro-alveograph (Tripette and Renaud, Paris, France) using 50 or 60 g of flour milled in a Chopin–Dubois or Brabender Quadrumat Senior mill respectively, and applying manufacturer's correction coefficients. Parameters recorded were deformation energy W, an index of gluten strength, measured in $J \times 104$; and the ratio of overpressure P (mm H_2O) to swelling index L (cm), an index of tenacity/extensibility. At INRA the two components of the latter ratio were also recorded.

Other tests were: measurement of cookie diameter (AACC-AM 10-52) in CA 99, AWRC in CA 95 and 99 by the method of Yamazaki et al. (1968), and

solvent retention capacity for lactic acid (AACC-AM 56-11) in CA 96. The latter is one of a suite of tests for soft-wheat quality (Guttieri et al., 2004) measuring gluten strength by ability of flour to retain various solvents after centrifugation. Loaf volume was measured at CIMMYT by rapeseed volume displacement with the straight-dough baking test 10-10 of the AACC (AACC, 1995).

Marker genotyping and analysis

The restriction fragment length polymorphism (RFLP) genotyping of the RI lines has been described in Nelson et al. (1995a) and Van Deynze et al. (1995). They were additionally scored via SDS-PAGE as described in William et al. (1993) for high-molecular-weight glutenin subunits. Map locations of gliadin and LMW glutenin loci not directly genotyped were inferred from linkage data in McIntosh et al. (2003) and other sources as described below.

QTL analysis of quality traits was based on 554 markers covering a total map distance of 3715 cM over the 21 wheat chromosomes, with an average interval of 7 cM. Calculations were performed with the software application QTL Cartographer (Wang et al. 2004) for simple and composite interval mapping using forward selection of two or five cofactors. Significance thresholds were established by permutation testing (Churchill & Doerge, 1994). For twice- replicated dough-strength assays in France and Mexico, averages over replicates were used.

In the hexaploid wheat genome, marker analysis permits the identification of the parental component contributing an increasing allele for a given trait. Alleles in A- and B-genome regions favoring the M6 parent derived from the TT parent and those in D-genome regions from the AeT parent, while the alternative alleles all derived from Opata.

Results

Trait statistics

Trait values were reasonably unimodal and symmetrically distributed except for the ratio *P/L*, whose distribution was somewhat skewed to the lower end. These near-normal distributions are consistent with some combination of quantitative genetic control and an environmental component. While neither Opata nor M6 is a high-quality pastry or breadmaking wheat, in general the Opata scores indicated a hard wheat with stronger, less stiff dough and lower protein than M6 (Table 1).

Because quality parameters were estimated by differing methods in different locations, using different subsets of the genetic population, we did not attempt to estimate heritability. Rough indications are given by correlations between scores across years and locations, supported by examination of the influential genomic regions common to these. In the following, "environment" is used to denote a single location irrespective of test year.

Correlations between traits

For assessment of linear correlation trends, traits were grouped into four classes: protein concentration, kernel texture, mixing parameters, and dough strength; and r values significant at $p \leq 0.05$ are presented in Table 2.

Protein correlations. Grain and flour protein correlations across years within environments were higher (California and France, $r \sim 0.7$) than those across environments ($r = 0.45$–0.67 except for Mexico where the range was from insignificance to 0.28). Within and across environments, protein concentration was in general positively correlated with grain hardness, with alveogram *L* and *W* parameters ($r = 0.28$–0.44), and with Zeleny sedimentation scores ($r = 0.27$–0.59) but poorly with Pelshenke scores. Protein concentration measured in all environments but Mexico was positively correlated with dough viscosity via mixogram measures of viscosity and work as trace width, value, and integration, and negatively correlated with time and slope scores. In Mexico, protein was moderately ($r = 0.40$) correlated with time to heading (data not shown).

Dough-strength correlations. Alveogram extensibility parameter *L* was negatively correlated with *P* ($r = -0.34$–0.57), with *P/L* as would be expected, and with hardness measures. *L* was not correlated with measures of gluten strength including *W*, SDS, Zeleny, Pelshenke, and lactic acid retention, which were highly positively intercorrelated across environments and were generally positively correlated with hardness. However the trend of positive correlation of *L* and strength with mixing properties was generally similar to that described above for protein. The *P/L* ratio was strongly correlated with *P* but rarely with any other measure except sometimes *W*.

Hardness correlations. Measures of kernel hardness showed correlations of $r = 0.41$–0.83 across environments and methods (where softness equivalent was

Table 1. Summary statistics of selected quality traits in ITMI lines

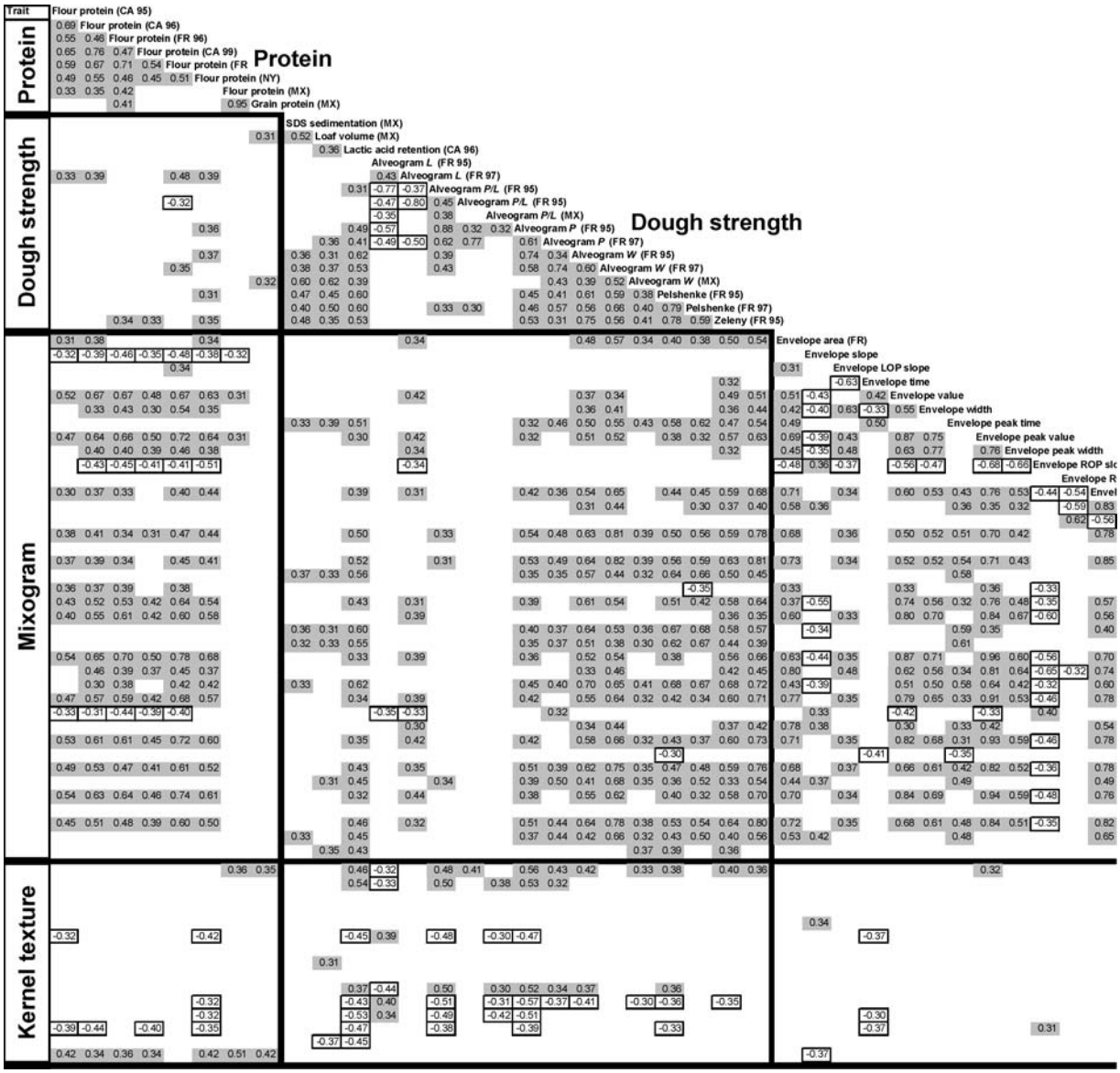
Class	Loc	Trait	Year	N	Mean	CV	Opata	Syn	Unit
Protein	CA	Flour protein	96	107	11.9	0.07	10.8	13.3	% wt
	CA	Flour protein	99	107	12.3	0.07	10.9	13.1	”
	FR	Flour protein	97	75	11.8	0.14	10.5	12.1	”
Dough strength	CA	Lactic acid retention	96	107	110.1	0.11	114.4	105.7	”
	FR	Alveogram L	97	75	89.1	0.26	101.0	104.0	cm
	”	Alveogram P/L	”	”	1.0	0.52	0.7	0.7	mm cm^{-1}
	”	Alveogram P	”	”	81.5	0.25	71.0	75.0	mm H_2O
	”	Alveogram W	”	”	202.5	0.29	210.0	210.0	$J \times 10^4$
	”	Pelshenke score	”	”	82.2	0.34	116.0	49.0	min
	”	Zeleny score	”	”	47.5	0.19	54.0	41.0	ml
Mixogram	”	Envelope area	”	”	51.9	0.19	53.6	67.3	% torque min
	”	Mid-LOP_area	”	”	58.1	0.35	80.5	31.6	”
	”	Midpeak area	”	”	113.4	0.20	135.4	86.2	”
	”	Mid-ROP area	”	”	224.8	0.12	246.2	200.0	”
	”	Mid-TIMX area	”	”	395.8	0.09	395.8	415.3	”
	”	Envelope LOP slope	”	”	5.9	0.76	10.1	0.8	% torque min^{-1}
	”	Mid-LOP slope	”	”	12.6	0.50	7.6	18.3	”
	”	Envelope ROP slope	”	”	−8.2	−0.32	−12.1	−8.9	”
	”	Envelope TIMX slope	”	”	−1.6	−1.00	−3.3	−0.5	”
	”	Mid-TIMX slope	”	”	−1.3	−0.40	−2.3	−0.4	”
	”	Envelope TAIL slope	”	”	−1.5	−0.65	−2.4	−1.1	”
	”	Mid-ROP slope	”	”	−2.3	−0.63	−1.6	−2.6	”
	”	Midtail slope	”	”	−0.8	−0.56	−1.4	−0.8	”
	”	Envelope LOP time	”	”	1.5	0.28	1.0	2.0	min
	”	Envelope peak time	”	”	2.3	0.23	3.1	2.3	”
	”	Midpeak time	”	”	2.6	0.18	3.1	1.9	”
	”	Envelope ROP time	”	”	5.0	0.23	4.3	4.7	”
	”	Envelope LOP value	”	”	70.6	0.10	64.9	77.3	% torque
	”	Mid-LOP value	”	”	50.9	0.11	51.2	47.6	”
	”	Envelope peak value	”	”	74.3	0.11	76.4	77.7	”
	”	Midpeak value	”	”	57.6	0.10	57.3	58.4	”
	”	Envelope TIMX value	”	”	53.8	0.11	54.8	56.2	”
	”	Mid-TIMX value	”	”	47.2	0.10	47.9	50.0	”
	”	Mid-ROP value	”	”	53.6	0.09	53.9	55.8	”
	”	Envelope ROP value	”	”	62.1	0.13	66.7	64.7	”
	”	Midtail value	”	”	45.1	0.11	44.6	48.0	”
	”	Envelope tail value	”	”	50.6	0.12	49.7	53.3	”
	”	Envelope LOP width	”	”	40.7	0.14	42.6	39.2	”
	”	Mid-LOP width	”	”	42.2	0.14	38.4	47.5	”
	”	Envelope peak width	”	”	36.9	0.15	37.3	39.0	”
	”	Midpeak width	”	”	32.3	0.18	37.0	39.5	”
	”	Envelope ROP width	”	”	20.1	0.32	22.8	23.2	”
	”	Mid-ROP width	”	”	20.6	0.19	20.6	25.0	”
	”	Midtail width	”	”	11.5	0.36	10.1	12.1	”
Kernel texture	CA	AWRC	99	107	70.3	0.11	75.8	70.4	% wt
	”	Flour yield	99	”	74.8	0.02	76.3	73.7	”
	”	Softness equivalent	99	”	35.9	0.16	33.7	34.8	”

Note: Traits are those for which both parents were also assayed. Values are from single assays except for dough-strength traits, where means of two sample assays are given

used, the sign of the correlation is reversed). Hardness was strongly correlated with AWRC and flour milling yield, but showed no clear pattern of correlation with mixing traits.

Mixogram correlations. The 37 mixogram traits measured in France displayed numerous strong intercorrelations. No well-separated correlation groups could be seen, though slope and time measurements of

Table 2. Pearson correlations (*r*) between quality trait scores assayed in ITMI lines



Note: Only correlations $r \geq 0.30$ are shown, all significant at $p \leq 0.05$. Negative values are boxed; positive are shaded. For compactness, only selected representative traits are shown.

dough development and tolerance tended to be poorly correlated with width, value, and area measurements of viscosity.

Comparison of QTL interval-mapping methods

Empirical genomewise significance thresholds corresponding to an expected type I error of $p = 0.05$ were established by permutation analysis with QTL Cartographer using 300, 500, or 1000 iterations. Thresholds ranged from 3.5–3.9 for CIM with default parameters including five marker cofactors chosen by stepwise regression, and 4.0–4.6 for CIM with 10 cofactors (determined for comparison but not used for analysis). Increasing cofactor number usually increased the number of "QTLs" detected. We used the

default cofactor number but reduced cofactors to two for the mixogram traits, which were measured on only 75 RILs. QTL profiles with SIM and CIM usually agreed qualitatively. We did not report QTLs found only in the New York data from only 56 RILs.

Results of QTL analysis of individual traits

Protein concentration

Protein concentration ranged from 9 to 20%, with the high extreme in the French sample. In the three northern environments a region on chromosome arm 6DS containing the *Gli-D2* gliadin locus (Figure 1e) consistently influenced grain and flour protein concentration, with the AeT homozygote showing from 0.75 to 2.0 more percentage units of protein. Less consistent effects were due to TT alleles on 7AS in California in 1996 and 1999, TT alleles on 5AL in FR_95, and Opata alleles on 2DS in FR_97. QTL effects are tabulated in Table 3.

In Mexico the largest effect ($r^2 \sim 0.27$) on both grain and flour protein was from a AeT allele on chromosome arm 2DS (Figure 1c). The RILs with this allele had 1.1% higher grain protein concentration and headed about 6 days later than the Opata homozygotes, and the phenotypic correlation between days to heading (data not shown) and protein concentration was ~0.4. A secondary influence ($r^2 \sim 0.15$) on grain protein was due to Opata alleles on 2AL.

Kernel texture traits

Hardness and SE. In all environments the 5DS region surrounding puroindoline gene marker *Xmta9* (Sourdille et al., 1996) showed strong ($r^2 \sim 0.37$–0.52) association with hardness or SE, with AeT contributing the softness allele (Figure 1d). The only other region influencing hardness (as SE) in more than one environment was near the centromere on 4D, with the Opata allele increasing softness in CA 95 and New York.

Flour yield. The *Ha-5DS* region accounted for respectively 46, 20, 20, and 15% of the variation in unadjusted flour yield for the France, New York, and two California samples, and no minor QTLs appeared consistently. The weight of overs from the 94-mesh sieve was increased mainly by the Opata *ha* allele for hardness ($R^2 = 0.21$), while total flour yield was increased by the *Ha* allele from AeT. The 40-mesh overs were increased by the *ha* allele in CA 95, but the New York data showed neither this effect nor any other. The *Ha* region was insignificant in both analyses of flour yield adjusted for softness.

AWRC and cookie diameter. The major association of these water-retention-related traits in both CA 95 and CA 99 was with the AeT *Ha* allele ($r^2 = 0.45$–0.59).

Dough strength

The correlation between Pelshenke and Zeleny measures was consistent with the sharing of genomic regions of influence. Effects seen in more than one environment were from Opata on 5DS near the *Ha* locus (Figure 1d) and from TT near the end of chromosome arm 1BS. Those seen in one environment were for Zeleny score on 1AS near *Gli-A1* (Opata), 2DS (Opata), and 5AL (TT). Lactic acid retention showed only the strong 5DS association. The correlation of SDS sedimentation with these measures was not reflected in the genetic analysis, with SDS-increasing alleles from Opata on 2DL and 3A near the centromere.

Dough viscosity as alveograph *W* was invariably increased by TT alleles on 1BS near the *Gli-B1* gliadin loci [and tightly linked *Glu-B3* LMW glutenin loci (Payne, 1987)] but the effect reached nominal significance only in France. Single-environment QTLs for France were found on 2DS and 5AL (Opata). The 3A centromere region influencing SDS sedimentation and an additional 7BL effect from Opata increased *W* in Mexico, but not in France.

Dough extensibility index *L* measured at France was increased in 1995 by Opata alleles near *Gli-A1*and *Gli-B1* (Figs. 1a, b), in both years by TT alleles on 7AS ($r^2 \sim 0.12$–0.20), and in 1997 by Opata alleles in the HMW *Glu-A1* region on 1AL. Dough tenacity index *P* was influenced strongly in both France assay years by TT alleles around *Gli-B1* on 1BS, and in 1997 by Opata alleles on 3BS ($r^2 \sim 0.21$). In France the ratio *P*/*L* of these two measures, considered to indicate dough elasticity, was influenced by loci that affected the components, being increased by TT alleles at the 1AS and 1BS QTLs and by Opata alleles at the 3BS QTL.

No reliable QTL associations were found for loaf volume as measured in Mexico.

Mixogram traits

U.S. data. In the U.S. samples, mixing time was most increased ($r^2 = 0.26$) by TT alleles on chromosome arm 1BS around gliadin locus *Gli-B1*. These alleles also strongly increased the tail widths of the mixogram trace, indicating positive effects on mixing tolerance. Correspondingly, Opata alleles near the 1BS gliadin locus increased tail slope, so that all positive effects on U.S.-measured dough quality from this genomic region originated in the durum parent. Aside from 1BS,

the only consistently influential genomic region was on chromosome 6A near the centromere, where the TT allele increased many mixogram-trace value, width, and area traits. Effects were found by CIM on 13 chromosomes in all, but with a sample size of 56 lines, we considered reliable only the 1BS effect supported by the French mixogram results.

French data. For the French mixogram data, TT effects on chromosome 1A originated near *Gli-A1* on mixing tolerance (as midtail slope) and near *Glu-A1*

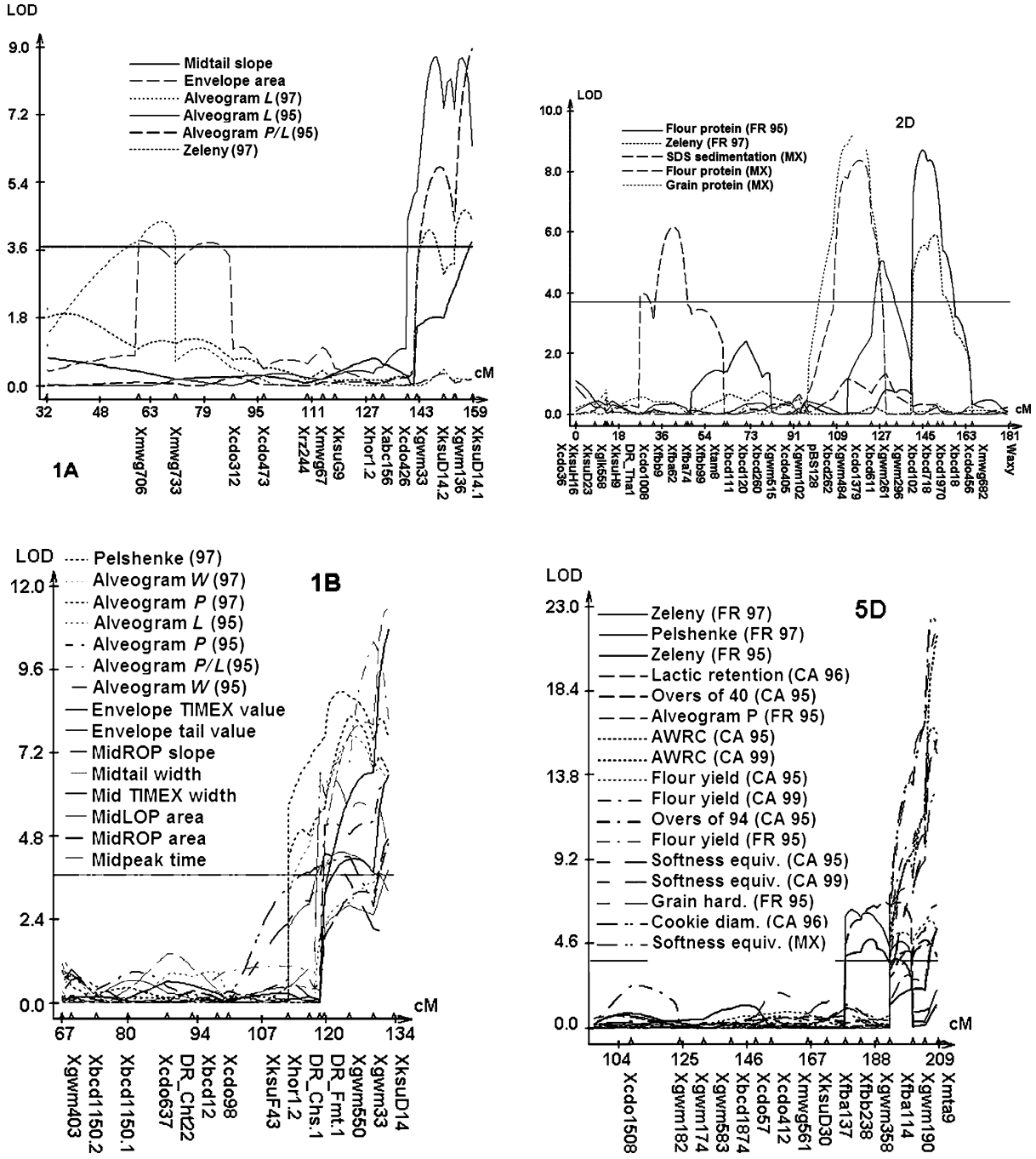


Figure 1. Composite-interval-mapping plots for putative quality QTLs in ITMI lines, on five chromosomes influencing the greatest numbers of quality traits. FR: France; MX: Mexico: CA: California. Traits without location labels were evaluated in France.

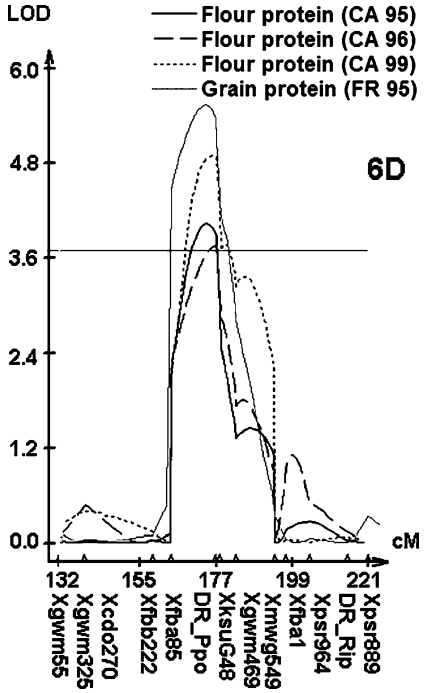


Figure 1. (*continued*)

on envelope ROP slope and two width or value traits to right of the mixogram peak, and near *Gli-B1* on some eight traits including tolerance (as mid-ROP slope) and viscosity (time, width and value). Opata alleles on 3AL and 7DL increased mid-ROP width and envelope peak time respectively, and on 5DS increased mid-LOP area, while AeT alleles near *Gli-D2* on 6DS increased area and value traits at and to right of the mixogram peak. The 5DS effect was from the same region near *Ha* where Opata alleles increased dough strength as measured by Zeleny and lactic-acid solvent retention.

Discussion

Statistical limitations of the study

The diversity of analytical methods, of subsets of ITMI lines used, and of replication within sites and over years used in the different locations and laboratories prevents a rigorous separation of contributions to variation in the quality traits. Reliably elucidating the genetic control of modestly heritable polygenic traits requires marker studies of much larger populations in multiple environments [*cf.* Kearsey and Farquhar (1998), Schön et al. (2004)] For this reason we attached most credence to QTL effects supported by prior knowledge and genetic evidence.

Protein concentration and heading time

Of interest is whether the locus influencing protein on chromosome arm 2DS is identical to the major earliness

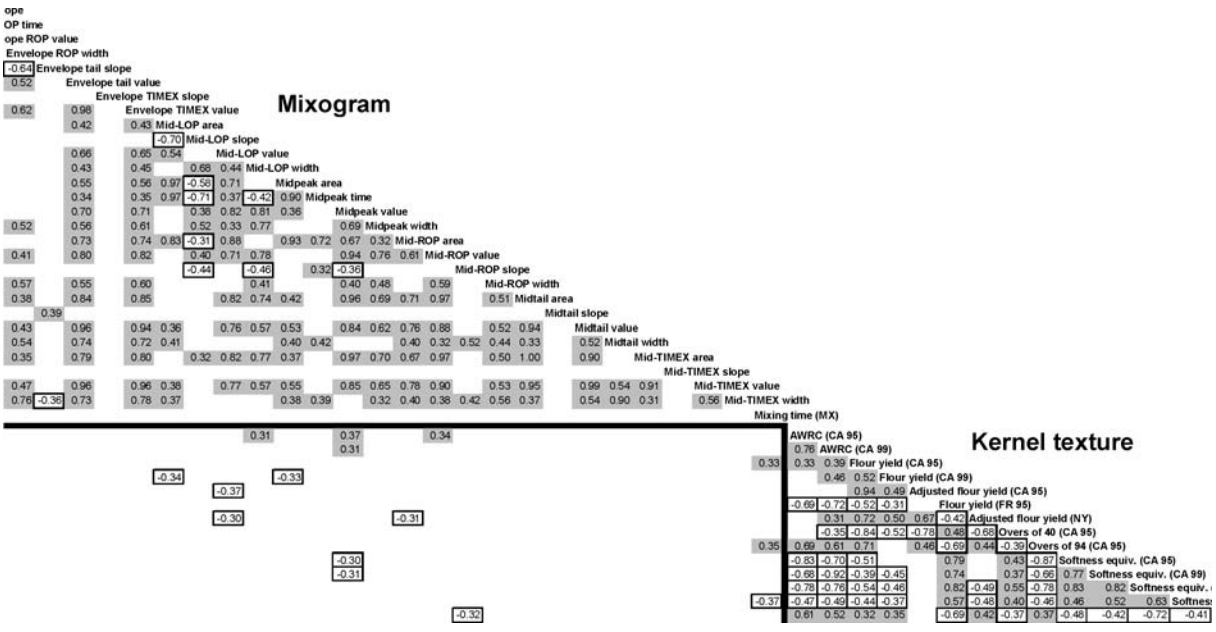


Table 3. Putative QTLs for quality traits found in ITMI lines

Character measured	Loc	Trait name	Yr	Chr	LOD	Add. effect	*R2*	Flanking markers
Extensibility	FR	Alveogram *L*	95	1A	8.8	18.6	0.3	*Xcdo426, XksuD14.1*
			97		4.4	10.3	0.19	*Xmwg706-Xmwg733*
			95	1B	3.7	11.2	0.11	*Xgwm550, XksuD14*
			95	3B	6.6	−14.2	0.19	*XksuH7, XgbxR080*
			95	7A	4.3	−12.1	0.12	*Xabc158, Xgwm60*
			97		4.6	−15.4	0.18	
Extensibility/viscosity	FR	Alveogram *P/L*	95	1A	8.8	−0.3	0.31	*Xcdo426, XksuD14.1*
			95	1B	10.4	−0.4	0.38	*Xgwm550, XksuD14*
Kernel texture	CA	Flour yield (adjusted)	95	4A	5.2	1.2	0.17	*XksuD9, Xcdo475*
	CA	Overs of 40	95		4.2	−10.7	0.13	*Xgwm63, Xwg622*
	CA	Lactic acid retention	96	4B	3.7	3.7	0.09	*Xbcd1265, Xbcd1051*
	CA	Flour yield (adjusted)	95	4D	3.8	1.1	0.14	*DR_Oxo1, Xcdo949*
	CA	Flour yield	95		4	1.3	0.16	
	CA	Overs of 40	95		3.9	−11.6	0.16	
	CA	Softness equivalent	95		5.8	2.6	0.15	*XksuF8, Xbcd265*
	CA	AWRC	95	5D	21.5	5.9	0.57	*Xgwm190, Xmta9*
			99		16.4	5.3	0.45	
	CA	Flour yield	95		5.9	1.4	0.2	
			99		4.5	0.7	0.15	
	CA	Overs of 40	95		4.8	−11.4	0.15	
	CA	Overs of 94	95		6.7	22.3	0.21	
	CA	Softness equivalent	95		15.8	−4.8	0.51	
			99		15.7	−4.2	0.52	
	FR	Flour yield	95		15.7	0.04	0.46	
	FR	Grain hardness	95		21.9	16.5	0.47	
	MX	Softness equivalent			13.1	−3.9	0.37	
	CA	Lactic acid retention	96		6.9	5.7	0.21	*Xfba137, Xfba114*
Protein concentration	MX	Grain protein		2A	4.1	0.4	0.15	*Xbcd152, Xfbb329*
	FR	Grain protein	95	2D	8.7	1.1	0.32	*Xbcd102, Xbcd18*
	MX	Flour protein			8.4	−0.7	0.25	*Xbcd611, Xcdo1379*
	MX	Grain protein			9.2	−0.6	0.27	
	FR	Grain protein	95	5A	6.9	−0.9	0.19	*Xcdo1312, Xabg391*
	CA	Flour protein	95	6D	4	−0.5	0.16	*Xfba85, Xgwm469*
			96		3.8	−0.3	0.09	
			99		4.9	−0.4	0.16	
	FR	Grain protein	95		5.5	−0.8	0.17	
	CA	Flour protein	96	7A	5.9	−0.4	0.16	*Xabc158, Xgwm60*
			99		4.6	−0.4	0.14	
Mixing stability	FR	Midtail slope	97	1A	3.8	−2.2	0.2	*Xcdo426, XksuD14.1*
	FR	MidROP slope	97	1B	4.6	−0.7	0.23	*Xgwm550, XksuD14*
Strength	FR	Zeleny	97	1A	4.7	3.3	0.14	*Xcdo426, XksuD14.1*
	FR	Alveogram *W*	97	1B	7.7	−31.9	0.28	*DR_Fmt.1, Xgwm550*
	FR	Pelshenke score	97		9	−16.7	0.32	
	FR	Alveogram *W*	95		4.8	−27.9	0.19	*Xgwm550, XksuD14*
	MX	SDS sedimentation		2D	6.2	1	0.21	*Xfbb9, Xfba62*
	FR	Zeleny score	97		5.9	3.9	0.18	*Xbcd102, Xbcd18*
	MX	Alveogram *W*		3A	4.3	21.7	0.13	*Xwg177, DR_Cht1*
	MX	SDS			4.3	0.8	0.11	
	MX	Alveogram *W*		3B	3.8	19.9	0.1	*Xabc174, Xgwm108*
	MX	Loaf volume			4.6	41.5	0.18	
	MX	Alveogram *W*		4A	3.9	21.6	0.12	*Xcdo1387, Xfba78*
	CA	Cookie diameter	96	5D	22.4	−0.8	0.59	*Xgwm190, Xmta9*
	FR	Zeleny score	97		5.4	3.8	0.16	
			95		4.9	3.4	0.15	*Xfba137, Xfba114*
	FR	Pelshenke score	97	6B	5.1	12.2	0.19	*XksuH4, Xcdo476*
	FR	Zeleny score	95	7A	5.8	−3.8	0.18	*Xfbb343, Xfba248*
	MX	Alveogram *W*		7B	4.5	23.1	0.14	*Xwg686, Xcdo686*
Tenacity	FR	Alveogram *P*	95	1B	6.3	−11.8	0.24	*Xgwm550, XksuD14*
			97		8.2	−12.3	0.35	
Viscosity	FR	Envelope_area	97	1A	3.9	6.1	0.2	*Xmwg706-Xmwg733*
					3.8	6.6	0.24	*Xmwg733-Xcdo312*
	CA	Lactic acid retention	96	1B	4.2	−4.5	0.13	*DR_Fmt.1, Xgwm550*
	FR	Envelope tail value	97		3.9	−4.4	0.21	*Xgwm550, XksuD14*
	FR	Envelope TIMEX value	97		6.6	−3.7	0.34	
	FR	Mid-LOP area	97		4.4	−11	0.26	*DR_Fmt.1, Xgwm550*
	FR	Midpeak time	97		6.6	−0.3	0.36	
	FR	Mid-ROP area	97		4.3	−14.5	0.26	
	FR	Midtail width	97		11.4	−2.8	0.46	*Xgwm550, XksuD14*
	FR	Mid-TIMEX width	97		10.8	−3.4	0.48	
	FR	Mid-ROP width	97	3A	4.4	2.6	0.22	*Xmwg961, Xbcd115*
	FR	Alveogram *P*	97	3B	7.6	10.2	0.26	*XksuH7, XgbxR080*
	FR	Mid-LOP value	97	4D	3.9	−2.4	0.19	*Xbcd1431, Xcdo1312*
	FR	Alveogram P	95	5D	5.5	10.7	0.2	*Xgwm190, Xmta9*

Note: "Loc": location, where FR = France, MX = Mexico, CA = California. "Yr": years, 1995 to 1999. "Add. effect": the estimated additive effect of substitution of a M6 (Synthetic) for an Opata QTL allele

gene *Ppd1*. This gene, conferring dominant insensitivity to short day length, has been incorporated in many cultivars of CIMMYT origin (Keim et al., 1973). The insensitivity allele enhances grain yield in both winter and spring wheats by permitting earlier heading under the short days of spring so that grain-filling can occur before heat and drought stress often associated with late summer (Pugsley, 1983; Worland et al., 1988; Sourdille et al., 2000, Li et al., 2002). The Opata allele at marker *Xbcd611* is associated with earlier heading in several environments, including New York (Li et al., 2002, and data not shown), and the gene responsible is most likely *Ppd1*. In fact, yield in Mexico was strongly correlated with presence of this allele (J.E. Autrique, unpublished data, 1994). Yield and protein concentration are commonly inversely correlated owing to competition between photosynthate accumulation and nitrogen metabolism. Noteworthy, however, is that two RILs showed both >14% protein and early heading in Mexico.

Storage protein associations with quality traits

Gliadins and LMW glutenins were the storage proteins showing most association with quality traits. HMW glutenins were not associated with variation in protein concentration and explained little of the variation in dough traits. By contrast, in different genetic material, Campbell et al. (2001) found *Glu-A1* associated with peak height, *Glu-B1* with peak height and mixograph tolerance, and *Glu-D1* with peak and mixing time, while Perretant et al. (2000) found *Glu-A1* to be associated with ALVW. Opata, with 13% grain protein, shows medium to strong dough. It presents the 2^*, 13+16, and 2 + 12 phenotypes respectively at the 1A, 1B, and 1D *Glu* loci, while the synthetic hexaploid parent carries 0 (null), 7 + 8, and 1.5 + T2 phenotypes at these loci. The 2^* band is considered superior to the 0 (Moonen et al., 1983). The parental alleles at the *Glu-B1* locus are considered to have about the same value for breadmaking quality, inferior to the preferred 17 + 18 allele (Payne, 1984). For *Glu-D1* the 2 + 12 band phenotype is considered to be associated with dough weakness, in contrast to the 5 + 10 pattern favored in material where it is present. The 1.5 + T2 phenotype was identified in *Ae. tauschii* (William et al., 1993) and no association with quality traits has been established. The deficit of desirable HMW glutenin alleles may account for the low association of this protein type with quality traits in the RILs as well as for the indifferent quality of the parents. It is well known (Weegels et al., 1996) that bread quality depends on an optimum combination of the two protein classes.

The 6DS markers associated with increased protein in all locations but Mexico lie close to the *Gli-D2* locus. This inference may be made from the position of *Gli-B2* on 6BS about 22 cM distal to the rRNA genes at the *Nor* locus (Dvorak & Chen, 1983) and 9 cM proximal to marker *XksuG48* on chromosome 6A (Marino et al., 1996). In a panel of European wheat cultivars, correlations approached $r = 0.5$ between protein concentration and the presence of certain gliadin SDS–PAGE bands (Branlard & Dardevet, 1985a), among them α and β gliadins, known (Wrigley & Shepherd, 1973) to be encoded by genes on the short arms of group-6 chromosomes. The increasing effect of the AeT allele is consistent with the approximately 2 higher percentage units of protein in M6 than in Opata in California and France where the QTL was detected. As for the 7AS QTL from TT, protein QTLs were reported on this arm by Campbell et al. (2001), Blanco et al. (2002), Groos et al. (2003), and Prasad et al. (2003), but only the last-named map is sufficiently comparable to the ITMI map used here to support a speculation that the QTLs were the same. At our protein QTL on 2DS distal to *Ppd1*, Börner et al. (2002) also reported a minor protein effect in the ITMI RILs but did not specify the parental source.

Gliadin or linked LMW glutenin loci on all three group-1 chromosomes were associated with dough-handling traits. The *Gli-A1* region influenced mixing tolerance and extensibility, the *Gli-B1* region influenced tolerance and viscosity, both *Gli-A1* and *Gli-B1* affected dough strength as alveograph *P*, and the *Gli-D2* region influenced mixogram width and value traits corresponding to a strong dough. Nieto-Taladriz et al. (1994) found high strength effects from *Gli-B1* bands in another recombinant inbred population, but other studies, including that of Branlard and Dardevet (1985a), found no correlation between gliadins and tenacity. Plainly gliadin bands, like other genetic markers, are predictive of quality scores only in certain genetic materials. The ratio *P/L* did not show consistent new marker associations beyond those detected in the components, suggesting that this derived character is of little value for selection.

Searching for common genetic factors underlying quality traits

The finding of no genetic and only inconsistent phenotypic association between kernel texture and protein

concentration echoes that of Symes (1965). That author, however, found particle size index, a measure of kernel hardness, to be a strong predictor of dough handling and loaf texture characteristics and, in view of this association, expressed pessimism about the improvement of soft wheat quality by breeding. In our study, as in that of Perretant et al. (2000), some association of dough handling traits with *Ha* appeared, although it is uncertain that the responsible locus is *Ha* itself.

The high phenotypic and marker-based correlations between mixogram measurements of dough viscosity suggest that, at least in this genetic material, most of the relevant quality information might be captured with marker assays for gliadin/LMW glutenin loci on chromosomes 1A, 1B, and 6D, and for *Ha* (measured by milling or with *PinA/PinB* assays) and associated strength QTLs on 5DS.

The durum parent made clear genetic contributions to dough mixing traits via the 1A and 1B regions. Durum wheats, though generally not used for leavened breads (but see Quick and Crawford, 1983), are also selected for dough strength. The enhancement of a dough viscosity parameter by the *Gli-D2* region from the *Ae. tauschii* accession suggests that other wild accessions harbor further genetic potential to improve quality, an inference supported by a study of backcross progeny of *Ae. tauschii* amphiploids to elite soft red winter wheats (Murphy et al., 1997). Several of the RILs were superior to either parent with respect to protein concentration and quality traits. In yield trials in California all but line 80, the highest-protein line, have yielded satisfactorily, although data (not shown) confirmed the generally expected negative correlation between protein level and yield.

Conclusions

Quantitative-trait locus analysis of wheat quality traits in a recombinant-inbred progeny yielded the following main findings:

1) The *Ha* hardness gene on chromosome arm 5DS was associated with variation in kernel texture, AWRC, and flour yield, all of which are related to the mechanics of kernel fracturing and starch damage during milling. This locus or linked loci also influenced dough strength and some mixing traits, with the *ha* (hard) parent contributing the increasing alleles.
2) Grain protein concentration, which varied widely across environments, was positively correlated with some measures of dough strength and was not associated with HMW glutenin loci. A consistent influence on this trait in the northern environments was attributed to *Ae. tauschii* alleles in the *Gli-D2* region. In Mexican-grown material, a locus in the region of photoperiod-insensitivity gene *Ppd1* accounted for 25% of variation in protein, with the *ppd1*-coupled allele associated with higher (1.1% by weight) protein concentration.
3) Dough strength according to the Pelshenke and Zeleny tests, while moderately correlated with viscosity as measured by mixograph, was strongly enhanced by Opata alleles at a 5DS locus near or identical to the *Ha* gene. Dough tenacity as alveogram *P* was increased by durum alleles on 1BS.
4) Gliadin or closely linked LMW glutenin loci but rarely HMW glutenin loci were associated with mixing properties. The bread-wheat parent Opata contributed 1AS effects on dough extensibility and viscosity and 5DS effects on viscosity, the durum parent of the synthetic enhanced strength and mixing tolerance via the *Gli-B1* region, and the *Ae. tauschii* parent increased viscosity traits via the *Gli-D2* region.
5) Some of the inbred lines from the cross showed quality ratings consistently superior to those of either parent.

This study provides a starting point for the dissection of environmental from genetic effects on the expression of genes involved in wheat milling and baking quality. While the results reaffirm that gliadin/LMW glutenin and hardness loci are among the key determinants of quality traits, only the use of a larger mapping progeny grown in more environments will reliably separate minor and environment-specific genetic effects from the more major and stable effects. Such an analysis could enable the development of a small subset of quality tests or marker assays having predictive power at least as high as any currently used suite of tests. There would then be promise in evaluating quality traits in intercross progeny of selected RI lines that carry opposite marker alleles or useful recombination events in a few well-defined regions. Of first interest may be the storage-protein loci, which in this study contributed useful alleles from diploid and tetraploid wheat relatives.

All data will be submitted to the GrainGenes database.

References

AACC. 1995. Approved Methods of the American Association of Cereal Chemists, 9^{th} ed. The Association, St. Paul, Minnesota.

Bassett, L.M., R.E. Allan & G.L. Rubenthaler. 1989. Genotype × environment interactions on soft white winter wheat quality. Agron J 81: 955–960.

Blanco, A., A. Pasqualone, A. Troccoli, N. Di Fonzo & R. Simeone. 2002. Detection of grain content QTLs across environments in tetraploid wheat. Plant Mol Biol 48: 615–623.

Börner, A., E. Schumann, A. Fürste, H. Cöster, B. Leithold, M.S. Röder & W.E. Weber. 2002. Mapping of quantitative trait loci determining agronomic important characters in hexaploid wheat (*Triticum aestivum* L.). Theor Appl Genet 105: 921–936.

Branlard, G. & M. Dardevet. 1985a. Diversity of grain proteins and bread wheat quality I. Correlation between gliadin bands and flour quality characteristics. J Cereal Sci 3: 329–344.

Branlard, G. & M. Dardevet. 1985b. Diversity of grain protein and bread wheat quality II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. J Cereal Sci 3: 345–354.

Campbell, K.G., C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, G. Hareland, R.G. Fulcher, M.E. Sorrells & P.L. Finney. 1999. Quantitative trait loci associated with kernel traits in a soft × hard wheat cross. Crop Sci 39: 1184–1195.

Campbell, K.G., P.L. Finney, C.J. Bergman, D.G. Gualberto, J.A. Anderson, M.J. Giroux, D. Siritunga, J. Zhu, C. Gendre, C. Roué, A. Vérel & M.E. Sorrells. 2001. Quantitative trait loci associated with milling and baking quality in a soft × hard wheat cross. Crop Sci 41: 1275–1285.

Campbell, W.P., C.W. Wrigley, P.J. Cressey & C.R. Slack. 1987. Statistical correlations between quality attributes and grain-protein composition for 71 hexaploid wheats used as breeding parents. Cereal Chem 64: 293–299.

Carrillo, J.M., M. Rousset, C.O. Qualset & D.D. Kasarda. 1990. Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alleles to quantitative traits. 1. Grain yield and quality prediction tests. Theor Appl Genet 79: 321–330.

Churchill, G.A. & R.W. Doerge. (1994) Empirical threshold values for quantitative trait mapping. Genetics 138: 963–971.

Cressey, P.J., W.P. Campbell, C.W. Wrigley & W.B. Griffin. 1987. Statistical correlations between quality attributes and grain-protein composition for 60 advanced lines of crossbred wheat. Cereal Chem 64: 299–301.

du Cros, D.L., L.R. Joppa & C.W. Wrigley. 1983. Two-dimensional analysis of gliadin proteins associated with quality in durum wheat: chromosomal location of genes for their synthesis. Theor Appl Genet 66: 297–302.

Dvorak, J. & K.C. Chen. 1984. Distribution of nonstructural variation between wheat cultivars along chromosome arm 6Bp: evidence from the linkage map and physical map of the arm. Genetics 106: 325–333.

Finney, P.L. & L.C. Andrews. 1986. Revised microtesting for soft wheat quality evaluation. Cereal Chem 63: 177–182.

Gaines, C.S., Kassuba, A. & Finney, P.L. 1996. Using the wire-cut and sugar-snap formula cookie test baking methods to evaluate distinctive soft wheat flour sets: implications for quality testing. Cereal Foods World 41: 155–160.

Groos, C., N. Robert, E. Bervas & G. Charmet. 2003. Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor Appl Genet 106: 1032–1040.

Guttieri, M.J., C. Becker & E.J. Souza. 2004. Application of wheat meal solvent retention capacity tests within soft wheat breeding populations. Cereal Chem 81: 261–266.

Igrejas, G., P. Leroy, G. Charmet, T. Gaborit, D. Marion & G. Branlard. 2002. Mapping QTLs for grain hardness and puroindoline content in wheat (*Triticum aestivum* L.). Theor Appl Genet 106: 19–27.

Kearsey, M.J. & A.G.L. Farquhar. 1998. QTL analysis in plants; where are we now? Heredity 80: 137–142.

Keim, D.L., J.R. Welsh & R.L. McConnell. 1973. Inheritance of photoperiodic heading response in winter and spring cultivars of bread wheat. Can J Plant Sci 53: 247–250.

Kerber, E.R. & K.H. Tipples. 1969. Effects of the D genome on milling and baking properties of wheat. Can J Plant Sci 49: 255–263.

Konzak, C.F. 1977. Genetic control of the content, amino acid composition, and processing properties of proteins in wheat. Adv Genet 19: 407–582.

Li, W.L., J.C. Nelson, C.Y. Chu, L.H. Shi, S.H. Huang & D.J. Liu. 2002. Chromosomal locations and genetic relationships of tiller and spike characters in wheat. Euphytica 125: 357–365

Mansur, L.M., C.O. Qualset, D.D. Kasarda & R. Morris. 1990. Effects of 'Cheyenne' chromosomes on milling & baking quality in 'Chinese Spring' wheat in relation to glutenin & gliadin storage proteins. Crop Sci 30: 593–602.

Marino, C.L., J.C. Nelson, Y.H. Lu, M.E. Sorrells, C.R. Lopes & G.E. Hart. 1996. Molecular genetic maps of the group 6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39: 359–366.

Martin, J.M., R.C. Frohberg, C.F. Morris, L.E. Talbert & M.J. Giroux. 2001. Milling and bread baking traits associated with puroindoline sequence type in hard red spring wheat. Crop Sci 41: 228–234.

McGuire, C.F. & F.H. McNeal. 1974. Quality response of 10 hard red spring wheat cultivars to 25 environments. Crop Sci 14: 175–178.

McIntosh, R.A., Y. Yamazaki, K.M. Devos, J. Dubcovsky, W.J. Rogers & R. Appels. 2003. Catalogue of Gene Symbols for Wheat. http://wheat.pw.usda.gov/ggpages/wgc/2003/

Mingeot, D. & J.M Jacquemin. 1999. Mapping of RFLP probes characterized for their polymorphism on wheat. Theor Appl Genet 98: 1132–1137

Moonen, J.H.E., A. Scheepstra & A. Graveland. 1983. The positive effect of the high molecular weight subunits 3 + 10 and 2* of glutenin on the bread-making quality of wheat cultivars. Euphytica 32: 735–742.

Murphy, J.P., C.A. Griffey, P.L. Finney & S. Leath. 1997. Agronomic and grain quality evaluations of *Triticum aestivum* × *Aegilops tauschii* backcross populations. Crop Sci 37: 1960–1965.

Nelson, J.C., A.E. Van Deynze, E. Autrique, M.E. Sorrells, Y.H. Lu, M. Merlino, M. Atkinson & P. Leroy. 1995a. Molecular mapping of wheat. Homoeologous group 2. Genome 38: 517–524.

Nelson, J.C., M.E. Sorrells, A.E. Van Deynze, Y.H. Lu, M. Atkinson, M. Bernard, P. Leroy, J.D. Faris & J.A. Anderson. 1995b. Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141: 721–731.

Nieto-Taladriz, M.T., M.R. Perretant & M. Rousset. 1994. Effect of gliadins and HMW and LMW subunits of glutenin on dough

properties in the F_6 recombinant inbred lines from a bread wheat cross. Theor Appl Genet 88: 81–88.

Olmos, S., A. Distelfeld, O. Chicaiza, A.R. Schlatter, T. Fahima, V. Echenique & J. Dubcovsky. 2003. Precise mapping of a locus affecting grain protein content in durum wheat. Theor Appl Genet 107: 1243–1251.

Payne, P.I., L.M. Jackson, E.A. Holt & C.N. Law. 1984. Wheat storage proteins: Their genetics and their potential for manipulation by plant breeding. Phil Trans Royal Soc Lond Ser B 304: 359–371.

Payne, P.I., K. Corfield, L.M. Holt & J.A. Blackman. 1981. Correlations between the inheritance of certain high molecular weight subunits of glutenin and bread-making quality in wheats of related pedigree. J Sci Food Agric 32: 51–60.

Payne, P.I., J.A. Seekings, A.J. Worland, M.G. Jarvis & L.M. Holt. 1987. Allelic variation of glutenin subunits and gliadins and its effect on breadmaking quality in wheat: analysis of F_5 progeny from Chinese Spring × Chinese Spring Hope 1A. J Cereal Sci 6: 103–118.

Peña, R.J., A. Amaya, S. Rajaram & A. Mujeeb-Kazi. 1990. Variation in quality characteristics associated with some spring 1B/1R translocation wheats. J Cereal Sci 12: 105–112.

Perretant, M.R., T. Cadalen, G. Charmet, P. Sourdille, P. Nicolas, C. Boeuf, M.H. Tixier, G. Branlard, S. Bernard & M. Bernard. 2000. QTL analysis of bread-making quality in wheat using a doubled haploid population. Theor Appl Genet 100: 1167–1175.

Pomeranz, Y. & P.C. Williams. 1990. Wheat hardness: its genetic, structural and biochemical background, measurements and significance. In: Y. Pomeranz (Ed.), Advances in Cereal Science and Technology, 10: 471–544. AACC, St. Paul.

Prasad, M., N. Kumar, P.L. Kulwal, M.S. Röder, H.S. Balyan, H.S. Dhaliwal & P.K. Gupta. 2003. QTL analysis for grain protein content using SSR markers and validation studies using NILs in bread wheat. Theor Appl Genet 106: 659–667.

Pugsley, A.T. 1983. The impact of plant physiology on Australian wheat breeding. Euphytica 32: 743–748.

Quick, J.S. & R.D. Crawford. 1983. Bread baking potential of new durum wheat cultivars. In S. Sakamoto (Ed.), Proc. 6th Int. Wheat Genet. Symp., pp. 851–856. Kyoto Univ., Kyoto, Japan.

Röder, M.S., V. Korzun, K. Wendehake, J. Plaschke, M.H. Tixier, P. Leroy & M.W. Ganal. 1998. A microsatellite map of wheat. Genetics 149: 2007–2023

Rousset, M., J.M. Carrillo, C.O. Qualset & D.D. Kasarda. 1992. Use of recombinant inbred lines of wheat for study of associations of high-molecular-weight glutenin subunit alleles to quantitative traits. 2. Milling and bread-baking quality. Theor Appl Genet 83: 403–412.

Schön, C.C, H.F. Utz, S. Groh, B. Truberg, S. Openshaw & A.E. Melchinger. 2004. Quantitative trait locus mapping based on resampling in a vast testcross experiment and its relevance to quantitative genetics for complex traits. Genetics 167: 485–498.

Sourdille, P., M.R. Perretant, G. Charmet, P. Leroy, M.F. Gautier, P. Joudrier, J.C. Nelson, M.E. Sorrells & M. Bernard. 1996. Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93: 580–586.

Sourdille, P., M.H. Tixier, G. Charmet, G. Gay, T. Cadalen, S. Bernard & M. Bernard. 2000. Location of genes involved in ear compactness in wheat (*Triticum aestivum*) by means of molecular markers. Mol Breed 6: 247–255

Symes, K.J. 1965. The inheritance of grain hardness in wheat as measured by the particle-size index. Aust J Agric Res 16: 113–123.

Van Deynze, A.E., J. Dubcovsky, K.S. Gill, J.C. Nelson, M.E. Sorrells, J. Dvorak, B.S. Gill, E.S. Lagudah, S.R. McCouch & R. Appels. 1995. Molecular-genetic maps for chromosome 1 in Triticeae species and their relation to chromosomes in rice and oats. Genome 38: 47–59.

Walker, A.E. & C.E. Walker. 1992. Documentation and Users Instructions for MIXSMART (Registered). Computerized Data Acquisition and Analysis for the Mixograph. National Mfg., Lincoln, Nebraska.

Wang, S., C.J. Basten & Z.-B. Zeng. 2004. Windows QTL Cartographer v2.0. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.

Weegels, P.L., R.J. Hamer & J.D. Schofield. 1996. Critical review: Functional properties of wheat glutenin. J Cereal Sci 23: 1–18.

William, M.D.H.M., R.J. Peña & A. Mujeeb-Kazi. 1993. Seed protein and isozyme variations in *Triticum tauschii* (*Aegilops squarrosa*). Theor Appl Genet 87: 257–263.

Worland, A.J., S. Petrovic & C.N. Law. 1988. Genetic analysis of chromosome 2D of wheat. II. The importance of this chromosome to Yugoslavian varieties. Plant Breed 100: 247–259.

Wrigley, C.W. & K.W. Shepherd. 1973. Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209: 154–162.

Yamazaki, W.T., J.R. Donelson & L.W. Briggle. 1968. Microtests for soft wheat quality evaluation. Crop Sci 8: 199–200.